REMARKS

Claims 1-7, 9-26 and 28 currently appear in this application. The Office Action of September 19, 2007, has been carefully studied. These claims define novel and unobvious subject matter under Sections 102 and 103 of 35 U.S.C., and therefore should be allowed. Applicant respectfully requests favorable reconsideration, entry of the present amendment, and formal allowance of the claims.

Rejections under 35 U.S.C. 112

Claims 1-14, 21, 22 and 24-26 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner alleges that "perfluorocarbon" is not adequately defined, and that "metabolizing nitric oxide" does not indicate what diseases are treated by controlling the metabolism of nitric oxide. This rejection is respectfully traversed.

The term "perfluorocarbon" is well known to those skilled in the art, particularly in the field of medicine. Submitted herewith are copies of the following:

Spahn, *Crit. Care* 1999 **3(5)** R93-R97;

Wikipedia, "perfluorocarbon";

F2 Chemicals Limited Core Product Range and Applications;

Leone, demsonline.org/jax-medicine/1998journals/december98/artifical blood.

In all of these articles "perfluorocarbon" is used to describe chemically inert synthetic molecules that consist primarily of carbon and fluorine atoms and are clear, colorless liquids (Spahn). While Spahn describes a number of compounds including perfluorooctyl bromide, perfluorodecylbromide, etc., it should be noted that these are "second generation" perfluorocarbons. Wikipedia defines "perfluorocarbon" as compounds derived from hydrocarbons by replacement of hydrogen atoms by fluorine atoms.

F2 chemicals describes perfluorocarbon as inert liquids and gases containing only fluorine and carbon atoms.

The claims recite that the perfluorocarbons used have a molecular weight of from about 400 to about 700 Dalton. This should be sufficient for one skilled in the art, without undue experimentation, to determine what specific perfluorocarbons can be used in the herein claimed method.

With respect to the phrase "metabolizing nitric oxide", claim 1 has been amended to recite the disease that are treated by controlling the metabolism of nitric oxide. The specification is replete with citations to references that

teach the role of nitric oxide in a variety of diseases and conditions, including the following:

Page 2, last line of paragraph 0005, Influenza A pneumonitis or intestinal and liver injury caused by *T. Gondii*;

Page 5, paragraph 0012, control of blood clotting and inhibiting coagulation;

Page 6, paragraphs 0013-0015, bone remodeling disorders;

Page 8, paragraph 0020, arterial and venous smooth muscle relaxation, inhibit platelet aggregation, activate guanylate cyclase;

Page 20, paragraph 52, vasodilation, anti-platelet aggregation.

Thus, it is well known that controlling nitric oxide synthesis can be used to treat a variety of diseases and conditions. The problem heretofore has been in the unpredictable rate of decomposition of S-nitrosothiols in physiological vehicles, which can occur in the presence of copper and other divalent metal ions and by enzymatic degradation. The perfluorocarbons used in the herein claimed method to catalyze NO oxidation *in vivo* or to inhibit nitric oxide activity do hot have this disadvantage. As stated ion the specification at page 23, paragraph 0070, administering

about 1% w/v perfluorocarbons sequesters circulating nitric oxide. Administering small amounts of perfluorocarbons, less than about 0.5% w/v/, accelerates NO oxidation and NO-donor formation.

Claim 28 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention,. The Examiner states that the term "perfluorocarbon" is of indeterminate scope. This rejection is respectfully traversed.

As noted above, the term "perfluorocarbon" is well accepted in medical parlance as relating to chemically inert synthetic molecules that consist primarily of carbon and fluorine atoms and are clear, colorless liquids. According to Spahn, cited above, there are many types of perfluorocarbons that have been used as blood substitutes. One skilled in the art, in this case, a physician, would be familiar with the term "perfluorocarbon" and would appreciate that it includes many fluorinated hydrocarbons that have the properties described in the Spahn article. Moreover, claim 28, because it is dependent upon claim 1, limits the perfluorocarbons to those having a molecular weight of from about 400 to about 700 Dalton.

In view of the above, it is respectfully submitted that the claims are now in condition for allowance, and favorable action thereon is earnestly solicited.

Respectfully submitted,

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Artificial Blood: What Is It? Will I Use It?

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Artificial Blood: What Is It? Will I Use It?

Since the 17th century, blood transfusions have been attempted to offset blood loss from trauma and childbirth, or as a therapeutic modality during leeching or bloodletting. Until the identification of isoagglutinating antibodies, however, transfusions were fraught with significant early complications. These early complications sparked interest in using hemoglobin as an oxygen carrier in plasma. Early trials of these solutions proved disastrous as well, with significant immediate complications resulting from infusions of stroma-free human hemoglobin solutions.[1] These complications were most often acute renal failure thought to be the result of direct hemoglobin nephrotoxicity.[2]

History Of Artificial Blood

Development of a hemoglobin-based blood substitute was pursued vigorously by the military as a means to have an oxygen-carrying plasma expander available for battlefield use. Despite research throughout the Vietnam War, a clinically effective blood substitute was unable to be developed.

During this era of blood substitute research in the 1960s, Dr. Leland Clark began experimenting with a class of compounds known as perfluorocarbons. Oxygen has approximately 100 times greater solubility in perfluorocarbon solutions than in plasma. As a result, the amount of oxygen dissolved in plasma may be sufficient to sustain life, without the need for RBC-contained hemoglobin to provide additional oxygen. The hydrophobic nature of these compounds necessitated further development of perfluorocarbon emulsions prior to considering these compounds for use as a plasma oxygen carrier.

The use of Pluronic 64 as an emulsifying agent for perfluorocarbons enabled the production of Fluosol by the Green Cross Corporation of Japan. Clinical trials with this perfluorocarbon, however, were disappointing. Fluosol was present only in low concentrations in the emulsion, and Pluronic 64 caused rare but significant complications when the emulsion was infused intravenously. Further development of emulsion technologies resulted in the production of compounds which utilized smaller chain perfluorocarbon molecules to more effectively emulsify the perfluorocarbons, allowing higher concentrations of active agent in the emulsion and thus higher oxygen carrying capabilities. The improved stability of the newer emulsions are vastly superior to the first generations of perfluorocarbons; current emulsions can be stored at 4°C for extended periods of time (months) without appreciable degradation of activity.

Physiology Of Oxygen Transport

Normal oxygen transport is primarily a function of erythrocyte-contained hemoglobin. The heme-iron moiety of the hemoglobin molecule allows binding and release of oxygen, dependent upon the partial oxygen tension to which the hemoglobin is exposed. The tetrameric structure of the protein portion allows hemoglobin to bind four oxygen molecules within binding pockets in each protein subunit.

Modification of the ability of oxygen to bind to hemoglobin occurs naturally. Hemoglobin oxygen interactions result in structural conformational changes to facilitate loading and unloading of oxygen in the pulmonary circulation and peripheral tissues, respectively. The efficiency of oxygen binding and release can

be altered by acid-base balance, the partial pressure of carbon dioxide, temperature, and 2,3-diphosphoglycerate. The resulting shift of the sigmoidal oxy-hemoglobin dissociation curve serves as a natural regulatory mechanism for the delivery of oxygen to tissues (Figure 1). In neutral pH, in the absence of any other modifying substances or conditions, the P50 of native hemoglobin outside of the RBC is 17 mmHg. Thus, the internal milieu of the RBC is critical to the effective delivery of oxygen to tissues.

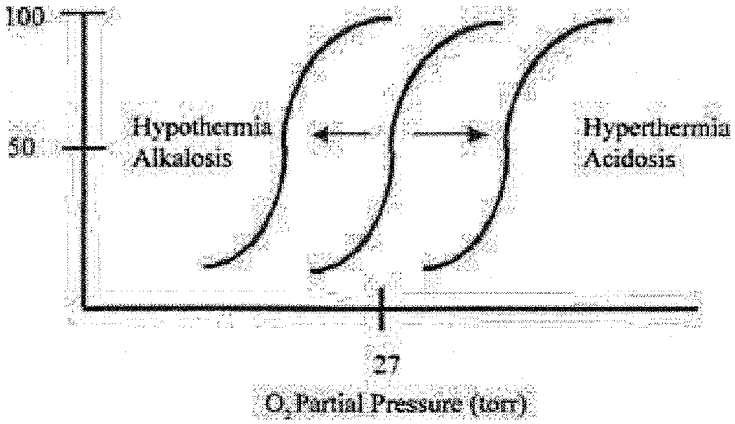

Figure 1. Oxy-hemoglobin dissociation may be affected by several conditions, including acidosis (high CO_2 levels), alkalosis (low CO_2 levels), hyperthermia and hypothermia.

Artificial blood solutions based on hemoglobin function by oxygen delivery from plasma hemoglobin. Initial trials of free hemoglobin solutions demonstrated little benefit to patients with these unmodified hemoglobin molecules, most likely due to the high affinity of oxygen for the plasma hemoglobin.[1, 3] Subsequent research has revealed several methodologies that are effective in altering the binding affinity of hemoglobin from oxygen in order to deliver oxygen to peripheral tissues. Ligands such as pyridoxyl groups, when bound to hemoglobin, alter the oxygen affinity, shifting the dissociation curve to the right. The decrease in oxygen affinity effected by these changes enables plasma hemoglobin to deliver oxygen to peripheral tissues.

Current Status Of Blood Substitutes

After many years of intensive research, blood substitute technology is finally reaching the point where safe, clinically effective solutions may become a reality. For perfluorocarbon emulsions, newer molecules, coupled with advances in emulsification technology, has produced solutions with great potential for clinical applications. Novel methods of crosslinking and chemical modification have made hemoglobin solutions a viable alternative as temporary oxygen carriers.

Perfluorocarbon Emulsions

After the initial excitement regarding Fluosol, subsequent small studies demonstrated no benefit from Fluosol infusions in patients with profound anemia.[4,5] With colloid solutions as a comparator, Fluosol did not improve indirect measures of oxygenation. However, Fluosol continued to be available for infusion as an oxygen carrier during high-risk percutaneous transluminal angioplasty procedures until early 1993, when approval for this indication for the emulsion was rescinded by the Food and Drug Administration.

New emulsions have been developed which utilize emulsifying agents similar to the primary compound. In particular, perflubron (perfluorooctyl bromide) has been developed as a stable emulsion safe for intravenous infusion by the addition of small amounts of perfluorodecyl bromide as an emulsifying agent; the emulsion is then buffered with egg yolk phospholipids. The resulting emulsion has a calculated oxygen carrying capacity which is approximately three fold the amount of oxygen carrying capacity of the earlier Fluosol

solutions.

Perflubron oxygen carrying capacity is directly related to the oxygen partial pressure (Figure 2). In this regard, perflubron oxygen delivery is predictable; direct diffusion of oxygen is the mechanism by which oxygen is off-loaded to peripheral tissues. Theoretically, oxygen delivered by diffusion may be more available, and more readily off-loaded from the bloodstream, than hemoglobin-delivered oxygen. However, no data have been produced which support this premise.

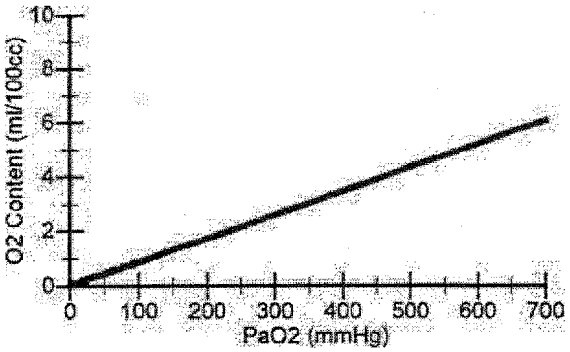

Figure 2. The oxygen content of perfluorocarbon emulsions obeys Henry's Law of partial pressures; the amount of oxygen dissolved in a perfluorocarbon solution is directly related to the partial pressure of oxygen to which the solution is exposed.

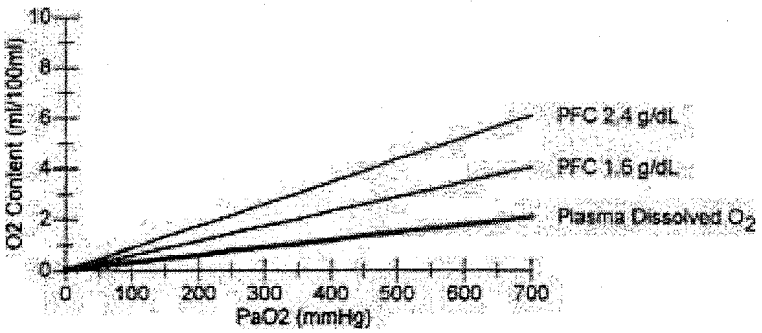

Figure 3. A comparison of the amount of oxygen dissolved in normal plasma and two clinically achievable plasma concentrations of perflubron.

Benefits Of Perfluorocarbon Oxygen Transport

Transport of oxygen as soluble gas in plasma is radically different from hemoglobin-based oxygen transport. Although some oxygen is normally dissolved in plasma, the amount typically constitutes less than 1% of the total oxygen content in arterial blood, even with significant anemia. By contrast, administration of perflubron can increase dissolved oxygen to approximately 10-15% of the total arterial oxygen content, an increase from the norm of two to three fold, depending on the partial pressure of oxygen inspired (Figure 3).

There is evidence to suggest that diffusion of oxygen does occur, and increased tissue oxygenation is the result. Studies on solid tumor treatment with either chemotherapy or radiation therapy have demonstrated enhanced tumor kill ratios when animals are pretreated with perflubron. Diffusion of oxygen into the hypoxic core of these tumors, thus spurring these "dormant" hypoxic tumor cells to divide, results in greater sensitivity of these now dividing tumor cells to antimitotic agents, enhancing their effectiveness.[6] This theory now awaits clinical trials to evaluate the efficacy of diffusion of oxygen into tissues.

Problems With Perfluorocarbons

Perfluorocarbons are inert biologically. The molecules are sequestered in the reticuloendothelial system, particularly in the Kupffer cells of the liver and macrophages, and subsequently released back into the plasma as a dissolved gas. The perfluorocarbon gas is then exhaled unchanged and non-metabolized via the lungs. While previous perfluorocarbons had a significant amount of retention in the reticuloendothelial system, current generation perfluorocarbons such as perflubron have a retention time of approximately one week. This allows effective elimination of perfluorocarbons from the liver and spleen without the potential for significant organ dysfunction.

However, despite the inert nature of perfluorocarbons, sequestration in the reticuloendothelial system may result in subtle consequences. Platelet count is known to decrease, presumably due to opsonization of platelets by the perfluorocarbon and subsequent sequestration and elimination by the reticuloendothelial

system. Sufficient perfluoro-carbon may also overwhelm the reticuloendothelial system, resulting in potential infectious or other complications; however, this is only a theoretical concern, as no increase in infectious complications has been noted in early clinical trials.

The retention of perfluorocarbons does pose an additional problem with respect to dosage. Perfluorocarbons are relatively evanescent in the plasma, with a half life of approximately 3-4 hours in the plasma phase. The reticuloendothelial system, however, has an approximate 3-5 day retention phase prior to exhalation of the perfluorocarbon. Therefore, although extremely short-lived in the plasma phase, additional dosing of perfluorocarbons may not be possible for several half-lives of the tissue-reticuloendothelial terminal elimination, i.e., one to two weeks. Thus perfluorocarbons become a single dose drug, with limitation of dosage due to the capacity of the reticuloendothelial system to handle the plasma elimination phase. At present, this limitation of dosing is theoretical, as no clinical data exist to discern whether perfluorocarbon redosing results in serious adverse effects; future studies and newer generation emulsions will address this issue.

The dependence of perfluorocarbons on Henry's Law of partial pressures allows the potential for increased oxygen availability. This fact of oxygen delivery also limits the effective use of perfluorocarbons to situations when the partial pressure of oxygen is supranormal, i.e., when the partial alveolar oxygen tension approaches 400 mmHg or greater. This is impossible to attain without supplemental oxygen administration; an effective partial oxygen pressure may be impossible with any maneuvers at altitude. Even in the presence of supplemental oxygen and controlled ventilation, patients with significant pulmonary disease may be unable to reach partial pressures of oxygen to allow perfluorocarbon to function as an effective oxygen carrier.

Hemoglobin-Based Oxygen Carriers

Stroma free hemoglobin has been produced for some time, yet significant renal toxicity has heretofore prevented its widespread use. Hemoglobin is a tetrameric protein of approximately 64,000 daltons; outside of its red blood cell milieu, the hemoglobin molecule rapidly dissociates into dimers composed of an alpha and a beta subunit. In addition to rendering the hemoglobin non-functional, these dimers are then filtered by the kidney, and the interaction of these hemoglobin residua with minute amounts of cell wall pieces in the renal glomeruli results in rapid acute tubular necrosis and renal failure. Development of a suitable stroma free hemoglobin molecule therefore depends on the development of a stable, functional tetramer of hemoglobin which would not dissociate into dimers upon infusion. This problem has been solved in several novel ways.

Prevention of dissociation of the hemoglobin tetramer in plasma is accomplished by binding the hemoglobin protein subunits together to prevent dissociation. Binding of the hemoglobin tetramer has been approached both chemically and genetically. Chemical binding of the tetramer involves binding of the alpha subunits by a so-called bifunctional agent, such as diaspirin, which links the hemoglobin molecules and thus stabilizes them. These polyhemoglobins are now undergoing clinical trials as potential blood substitutes.

A second significant problem is the lack of 2,3-DPG associated with the stroma free hemoglobin; the resulting stroma free hemoglobin, although polymerized with bifunctional agents, will not be functional at physiologic levels of tissue oxygenation. The P50 of native stroma free hemoglobin in solution is approximately 17 mmHg. This has been overcome chemically by the binding of pyridoxal phosphate to the hemoglobin molecule. The resulting polymerized, pyridoxi-lated stroma free hemoglobin has a P50 of approximately 32 mmHg (as compared to native, RBC associated hemoglobin P50 of approximately 27 mmHg) (Figure 4). Therefore, chemically altered stroma free hemoglobin are functionally superior to native hemoglobin.

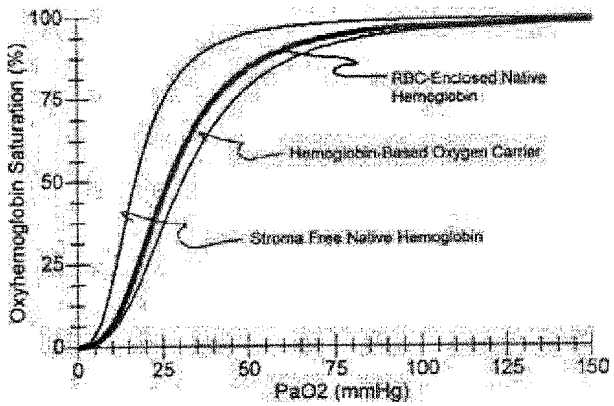

Figure 4. A comparison of the oxy-hemoglobin dissociation curves of native ("wild-type" or A1) hemoglobin contained with the normal red blood cell milieu ("RBC-Enclosed Native Hemoglobin"), native or "wild-type" hemoglobin after removal from a red blood cell ("Stroma Free Native Hemoglobin"), and typical hemoglobin based oxygen carrier solutions.

Another approach to hemoglobin modification has been genetic engineering. The structure and amino acid sequence of wild-type hemoglobin is known. Therefore, by genetically altering the native hemoglobin by the addition of a single amino acid, it is possible to covalently bind two alpha subunits, thus preventing the dissociation of the hemoglobin tetramer. A single point mutation in the beta subunits produces a hemoglobin with a P50 of approximately 32 mmHg. Thus specific mutations in the hemoglobin molecule result in a functional, stable stroma free hemoglobin. Insertion of this engineered hemoglobin into *E. coli* plasmids results in the production of large quantities of hemoglobin.[7] Purification of the hemoglobin would be similar to those processes used currently for other genetically engineered substances, such as insulin.

Xenograft material can also be used to produce stroma free hemoglobin. Bovine hemoglobin can be used after polymerization, as bovine hemoglobin does not require 2,3-DPG or other ligands to modify its oxy-hemoglobin dissociation.[8] A ready supply of this hemoglobin is available, and chemical sterilization of this protein possible, although the prospect of zoonotic infection must be considered, particularly with concern for prion disease.

Benefits Of Hemoglobin-Based Oxygen Carriers

All blood substitutes utilizing chemical sterilization involve the reclamation of human blood cells from outdated red blood cell products. Questions regarding the ability to chemically sterilize these products sufficiently to avoid infectious disease transmission have been largely answered; however, production of this product involves a ready supply of outdated blood in a time when voluntary donations are decreasing. Genetically produced hemoglobin from *E. coli* does not suffer from supply problems associated with the use of polymerized human hemoglobin. The use of bovine hemoglobin should be in ready supply theoretically for the foreseeable future. These approaches may prove more effective in satisfying the predicted high demand for these products.

Use of these products is predicated on knowledge of the serum half-lives of these preparations. In general, poly-hemoglobin preparations will increase in plasma half-life as their size is enlarged; a limit to the size is the viscosity and oncotic effects of the larger hemoglobin molecules. Most preparations will be retained in the plasma for half-lives of 8-30 hours.

These hemoglobin products, however, do not require a supraphysiologic oxygen tension to be effective in

delivering oxygen. Indeed, these compounds will most likely be more effective than native hemoglobin in delivering oxygen to tissues at physiologic arterial oxygen tensions (Figure 4). Thus hemoglobin-based oxygen carriers have an advantage over perfluorocarbons in this respect.

Hemoglobin-based oxygen carriers have some advantages over allogeneic red blood cell transfusions. The lack of iso-agglutinating antigens, due to the absence of a red cell membrane, obviates blood typing and screening and eliminates the most common morbidity and mortality of allogeneic and autologous transfusions, mismatching of blood units and the transfusion recipient. The lack of cross-matching requirements also allows virtually immediate availability of an oxygen carrier in critical periods of trauma or hemorrhage. However, there may be issues with administration of free hemoglobin in potentially septic situations.[9]

Disadvantages Of Hemoglobin-Based Oxygen Carriers

Plasma hemoglobin is not a true blood substitute; hemoglobin can replace only the oxygen transport capacity of whole blood, without the coagulation or immunologic aspects normally present in blood. While allogeneic blood may not supply these functions either, the plasma half-life of cross-matched allogeneic red blood cells is several fold greater than that of plasma hemoglobin. Thus, hemoglobin-based oxygen carriers will not replace blood, allogeneic whole blood, or allogeneic red blood cells completely. Thus use of these products may be limited to specific applications or in conjunction with specialized techniques, such as cardiopulmonary bypass with extracorporeal circulation or acute normovolemic hemodilution with harvesting of autologous whole blood for later reinfusion.

Free hemoglobin avidly binds nitric oxide. It is unknown whether this *in vivo* binding is of clinical significance, although binding of nitric oxide has been implicated as the cause of hypertension commonly seen with hemoglobin infusion. It remains to be determined what effects stroma free hemoglobin has on regional autoregulation of blood flow, and whether the hypertension associated with hemoglobin infusion has pathophysiologic consequences. At present, little data are available in large animal or clinical studies utilizing these compounds to elucidate the importance of this phenomenon.

Metabolism of plasma free hemoglobin-based oxygen carriers is identical to native hemoglobin released as a red blood cell is destroyed. Bilirubin levels will rise as hemoglobin is metabolized. Amylase levels also rise and some degree of lipase increase occurs; the pancreas appears to be the source of these increases in amylase and lipase, although no clinical evidence of pancreatitis has been documented in patients receiving hemoglobin-based oxygen carriers. The administration of these hemoglobin thus may cause significant alterations in laboratory values, potentially masking serious clinical consequences. Additionally, the consequences of metabolism of hemoglobin-based oxygen carriers may be similar to those of multiple transfusions, namely hemosiderosis and chronic iron overload.

Clinical Utility Of Blood Substitutes

Current blood substitutes have been demonstrated to be safe when administered in small quantities to volunteers. Both perfluorocarbon and hemoglobin based oxygen carriers have undergone clinical trials designed to determine the safety of these compounds when given to otherwise healthy patients. These preliminary studies have shown that a clinical useful dose of a blood substitute can be infused to patients. However, further information regarding the effectiveness and clinical usefulness of these compounds is in short supply at present.

The short plasma half-life of these compounds limits the usefulness of blood substitutes to short periods of time. Ultimately, the blood substitute will be sequestered or metabolized, and decreased oxygen carrying capacity will reappear as the plasma oxygen carrying capacity diminishes. Thus, if no longer acting agents are available, it is likely that these blood substitutes will merely delay an allogeneic transfusion, rather than

avoiding exposure, when used in place of conventional allogeneic red blood cell transfusions.

In order to effectively use these compounds, special techniques should be considered. One technique which theoretically should optimize blood substitute utility is acute normovolemic hemodilution. Aggressive harvesting of potentially several units of autologous fresh whole blood is possible when the solution to replace the harvested blood is capable of transporting oxygen. Coupling of blood substitutes with acute normovolemic hemodilution has been successful in small clinical trials; whether this mode of using blood substitutes will result in substantial clinical and economic benefits await larger clinical trials.

Summary

Blood substitutes are currently undergoing preliminary clinical trials to determine their safety. Two distinctly different classes of oxygen carriers are being developed, each capable of transporting and delivering oxygen to peripheral tissues. The delivery of oxygen by these two methodologies may have both benefits and risks which are unique to its class. Early clinical trials have been promising; however, effective use of these blood substitutes may involve using them in conjunction with other techniques such as normovolemic hemodilution to effectively reduce or eliminate the need for transfusions in certain instances. However, this first generation of clinically safe blood substitutes will not replace allogeneic blood transfusions as a means of treating many types of anemia.

References

1. Amberson WR, Jennings JJ, Rhode CM. Clinical experience with hemoglobin-saline solutions. *American Journal of Physiology*. 1949;1:469-489.
2. Lee R, Atsumi N, Jacobs EEJ, Austen WG, Vlahakes GJ. Ultrapure, stroma-free, polymerized bovine hemoglobin solution: evaluation of renal toxicity. *Journal of Surgical Research*. 1989;47:407-411.
3. Amberson W, Flexner J, Steggerda FR, et al. On the use of Ringer-Locke solutions containing hemoglobin as a substitute for normal blood in mammals. *Journal of Cellular and Comparative Physiology*. 1937;5:359-382.
4. Tremper KK, Lapin R, Levine E, Friedman A, Shoemaker WC. Hemodynamic and oxygen transport effects of a perfluorochemical blood substitute, Fluosol-DA (20%). *Critical Care Medicine*. 1980;8:738-741.
5. Tremper KK, Friedman AE, Levine EM, Lapin R, Camarillo D. The preoperative treatment of severely anemic patients with a perfluorochemical oxygen-transport fluid, Fluosol-DA. *New England Journal of Medicine*. 1982;307:277-283.
6. Teicher B. Use of perfluorocarbon emulsions in cancer therapy. In: Chang TMS, ed. *Blood Substitutes and Oxygen Carriers*. New York: Marcel Dekker, Inc 1993;640-647.
7. Hoffman SJ, Looker DL, Roehrich JM, et al. Expression of fully functional tetrameric human hemoglobin in *Escherichia coli*. *Proceedings of the National Academy of Sciences, U.S.A.* 1990;87:8521-8525.
8. Laver MB, Jackson E, Scherperel M, Tung C, Tung W, Radford EP. Hemoglobin-O2 affinity regulation: DPG, monovalent anions, and hemoglobin concentration. *J Appl Physiol*. 1977;43:632-642.
9. White CT, Murray AJ, Smith DJ, Greene JR, Bolin RB. Synergistic toxicity of endotoxin and hemoglobin. *Journal of Laboratory and Clinical Medicine*. 1986;108:132-137.

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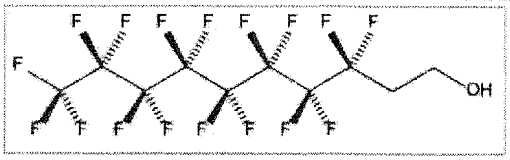
Perfluorocarbon

From Wikipedia, the free encyclopedia

Perfluorocarbons (PFCs) are compounds derived from hydrocarbons by replacement of hydrogen atoms by fluorine atoms. PFCs are made up of atoms of carbon, fluorine, and/or sulfur.



Fluorotelomer alcohol FTOH 8:2

Contents

Medicine

Perfluorocarbons are commonly used in eye surgery as temporary replacements of the vitreous humor in retinal detachment surgery. The length of the perfluorated carbon chain determines the physical properties of a particular perfluorocarbon. Small chain perfluorocarbons, such as perfluoro-propane, are gases that rise inside the eye and seal retinal holes. Larger chain perfluorocarbons, such as perfluoro-octane, are liquids heavier than water and are used in surgery to immobilize an infolded retina.

Perfluorocarbons are also used in contrast-enhanced ultrasound to improve ultrasound signal backscatter. The perfluorocarbons used in the microbubbles of some ultrasound contrast media are liquids at room temperature, but gases at body temperature. The gas-filled microbubbles oscillate and vibrate when a sonic energy field is applied and characteristically reflect ultrasound waves. This distinguishes the microbubbles from surrounding tissues.

Their stability, inertness, low diffusion rate and solubility increase the duration of contrast enhancement as compared to microbubbles containing air. The uses of PFCs in artificial blood and liquid breathing are currently being investigated. The theory behind liquid breathing is that, since perfluorocarbons carry oxygen so well, a person should be able to breathe normally with lungs filled with perfluorocarbon fluids. However, in practice liquid breathing has so far proved to be problematic; trials on animals have shown evidence of lung damage and carbon dioxide build-up from respiration. In addition, as perfluorocarbon liquids (and liquids in general) are much denser and more viscous than air, rates of breathing, and therefore of gas exchange, are limited.

Industry and the environment

PFCs are being used in refrigerating units and "clean" fire extinguishers. However, PFCs are extremely potent greenhouse gases, and they are a long-term problem with a lifetime up to 50,000 years (PMID 14572085). In a 2003 study, the most abundant atmospheric PFC was tetrafluoromethane (PMID 14572085). The greenhouse warming potential (GWP) of tetrafluoromethane is 6,500 times that of carbon dioxide, and the GWP of hexafluoroethane is 9,200 times that of carbon dioxide.[1] Several

governments concerned about the properties of PFCs have already tried to implement international agreements to limit their usage before it becomes a global warming issue. PFCs are one of the classes of compounds regulated in the Kyoto Protocol. Larger PFC compounds include PFOS and PFOA, which are persistent in the environment and are detected in blood samples all over the world.

See also

- Fluorinert - Coolants manufactured by 3M, some of which may contain PFCs
- Fluoropolymer
- Liquid breathing

External link

- Perfluorocarbons as a transport for Oxygen to the lungs, e.g. partial liquid ventilation.
- Envionmental Working Group defines PFC health concerns.

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Blood substitutes Artificial oxygen carriers: perfluorocarbon emulsions

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Abstract

Perfluorocarbon emulsions are being clinically evaluated as artificial oxygen carriers to reduce allogeneic blood transfusions or to improve tissue oxygenation. Perfluorocarbon emulsions are efficacious in animal experiments, and in humans they are well tolerated and at least as successful to reverse physiologic transfusion triggers than autologous blood. Perfluorocarbon emulsions may be used in the future in the concept of augmented acute normovolaemic haemodilution. In this concept relatively low preoperative haemoglobin levels are targeted during preoperative normovolaemic haemodilution and a perfluorocarbon emulsion is given to augment oxygen delivery during surgery when low endogenous haemoglobin levels are expected. The autologous blood is subsequently retransfused in the postoperative period when the patient's oxygenation is provided primarily by the endogenous haemoglobin. Additional uses of perfluorocarbon emulsions will include treatments of diseases with compromised tissue oxygenation such as cerebral or myocardial ischaemia, air embolism and emergency or trauma surgery as long as no allogeneic blood is available.

Keywords: acute normovolaemic haemodilution, artificial oxygen carriers, blood conservation, blood transfusion, perfluorocarbon emulsion

Introduction

Artificial oxygen carriers aim at improving oxygen transport and oxygen unloading to the tissue. Artificial oxygen carriers may thus be used as an alternative to allogeneic blood transfusions or to improve tissue oxygenation and function of organs with marginal oxygen supply. The present article describes the currently evaluated perfluorocarbon emulsions, in order to summarize their efficacy, discuss potential side effects and illustrate potential future applications.

Background of perfluorocarbon emulsions

Perfluorochemicals are chemically inert synthetic molecules that consist primarily of carbon and fluorine atoms, and are clear, colourless liquids. They have the ability to physically dissolve significant quantities of many gases including oxygen and carbon dioxide. Perfluorochemicals are hydrophobic, and are therefore not miscible with water. Perfluorochemicals thus have to be emulsified for intravenous use. With sophisticated technology, it is possible to generate a stable perfluorocarbon emulsion with exceptionally small particles (median diameter < 0.2 μm) [1].

First-generation perfluorocarbon emulsions as well as Russian or Chinese products have been described in a previous review [1]. Western development of second-generation perfluorocarbon emulsions has focused on linear perfluorocarbon molecules such as perfluorooctyl bromide

(C8F17Br, also referred to as perflubron), perfluorodecyl bromide (C10F21Br) and perfluorodichlorooctane (C8F16Cl2) [1]. Using solely 3.6%w/v egg yolk phospholipid, Alliance Pharmaceutical Corporation (San Diego, California, USA) developed a concentrated (60% w/v) and stable perfluorocarbon emulsion, Oxygent, which is composed of 58% w/v perfluorooctyl bromide and 2% w/v perfluorodecyl bromide, with an average particle size of 0.16-0.18 μm diameter. Hemagen/perfluorocarbon (St Louis, Missouri, USA) developed another concentrated (40% v/v) perfluorocarbon emulsion, Oxyfluor, using perfluorodichlorooctane, egg yolk phospholipid and triglycerid with an average particle size of 0.22-0.25 μm diameter.

After intravenous administration, the droplets of the perfluorocarbon emulsion are taken up by the reticuloendothelial system (RES). This uptake into the RES determines the intravascular half life [1,2,3]. Intravascular half life is dose-dependent, and for Oxygent was found to be 9.4 ± 2.2 h for a dose of 1.8 g/kg [4]. After the initial uptake of the perfluorocarbon emulsion into the RES the droplets are slowly broken down, and the perfluorocarbon molecules are taken up in the blood again (bound to blood lipids) and transported to the lungs, where the unaltered perfluorocarbon molecules are finally excreted via exhalation. At present no metabolism of fluorocarbon molecules is known in humans [1,2,3].

Safety profile

Oxygent and Oxyfluor both underwent extensive safety testing. The results of most of these studies are not published in the scientific literature, but they form the basis for regulatory authorities to allow clinical testing. Common side effects such as delayed febrile reaction and flu-like symptoms seem to be related to the normal activity of phagocytic cells of the RES. The magnitude of these side effects depend on the particle size of the emulsion. Smaller particles (0.1-0.2 μm diameter) appear to be less detectable for the RES than larger particles (> 0.2 μm diameter) and cause only mild temperature increases and mild flu-like symptoms in relatively few individuals [1,3,5].

Once in the RES, excretion depends on vapor pressure and lipid solubility of flourocarbons. Elimination half-time is 3-4 days for perfluorooctyl bromide and 8 days for perfluorodichlorooctane. Because flourocarbon molecules are inert to biochemical degradation, they diffuse back into the blood where they dissolve in plasma lipids. These lipids transport the perfluorocarbon molecules to the lungs where they are excreted by exhalation.

At higher doses (1.7 g/kg Oxyfluor, 1.8 g/kg Oxygent) a transient decrease in platelet count was observed 2-3 days after dosing, with recovery by 7-14 days [1,6]. With Oxygent, however, the decrease in platelet count was mild (10-20 %) and no drug-related effects on platelet function were observed. Furthermore, plasmatic coagulation was not compromised and template bleeding time was not prolonged by 1.8 g/kg Oxygent [1,6]. In addition, there was no complement activation [7]; no suppression of humoral or cell-mediated immune function; no haemodynamic effects or vasoconstriction; no changes in liver, lung or renal function; and no clinically relevant effects on blood chemistry [1].

Oxygen transport

Oxygen transport characteristics of perfluorocarbon emulsions are fundamentally different from those of blood (Fig. 1). Blood exhibits a sigmoidal oxygen dissociation curve. In contrast, perfluorocarbon emulsions are characterized by a linear relationship between oxygen partial pressure and oxygen content. Elevated arterial oxygen partial pressures are thus beneficial to maximize the oxygen transport capacity of perfluorocarbon emulsions. Ventilation with 100% oxygen may raise concerns regarding oxygen toxicity. During relatively short exposure times (< 8 h), however, no evidence of oxygen toxicity was detected [8] and the earliest signs of oxygen toxicity were observed only after 18h [9].

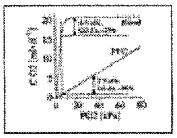

Figure 1

Perfluorocarbon emulsion and blood-based oxygen transport: oxygen dissociation curve of native human blood (Blood) and a perfluorocarbon (PFC) emulsion. Note that 5 volume % (5 Vol%) of oxygen can be offloaded by blood as well as by a PFC emulsion. (more ...)

Red blood cells are flexible, disk-shaped cells approximately 7-8 μm in diameter and are packed with highly concentrated hemoglobin. In arterioles red cells fill most of the vessel diameter with a relatively small plasma phase near the vessel wall where smaller cells such as platelets (1 μm in diameter) and small particles concentrate (near-wall particle excess phenomenon). In the capillaries the distance between red blood cells increases, producing significant intercellular plasma gaps, and capillaries are found that are perfused by plasma only.

Due to the small size (< 0.2 μm in diameter) perfluorocarbon emulsion particles mainly flow in the peripheral plasma layer in larger vessels [1,10]. In the microcirculation, perfluorocarbon emulsion particles perfuse even the tiniest capillaries (4-5 μm in diameter), where no red blood cells may flow under certain conditions. It is precisely this area in which perfluorocarbon emulsions exert their greatest effects, because they augment local oxygen delivery much more than would be expected from the increase in oxygen content in the arterial blood (large vessel with red blood cells) [1]. Another important aspect that determines the efficacy of perfluorocarbon emulsions is the fact that all oxygen carried by the perfluorocarbon is in the dissolved state, resulting in a higher oxygen partial pressures in the microcirculation and thereby augmenting the driving pressure for the diffusion of dissolved oxygen into the tissue.

Preclinical efficacy of second-generation perfluorocarbon emulsions

A perflubron emulsion (Oxygent) was assessed in a variety of haemodilution studies. Keipert *et al* [11] applied perflubron emulsion in dogs after acute normovolemic haemodilution (ANH) at a haematocrit of 10%. With the application of Oxygent, cardiac output tended to increase and a massive rise in mixed venous oxygen partial pressure and mixed venous saturation was observed. The percentage of metabolized oxygen that originated from endogenous haemoglobin decreased with the application of Oxygent, indicating that the oxygen transported by Oxygent is preferentially metabolized, due to its excellent oxygen unloading characteristics [11].

An increase in mixed venous oxygen partial pressure indicates improvement in global oxygenation status [12], rather than shunting of oxygen from arterioles directly into venules (i.e. oxygen bypassing the microcirculation). This view is substantiated by a study that demonstrated an increase of oxygen consumption in a maximally working, in-situ gastrocnemius muscle preparation in anesthetized and haemodiluted dogs after Oxygent administration [12]. It is also substantiated by the fact that increases in mixed venous oxygen partial pressure are generally associated with an Oxygent-dependent improvement in tissue oxygenation, as demonstrated in the heart, brain, muscle, gut and liver [13,14]. Furthermore, Holman *et al* [15] tested Oxygent in severely haemodiluted dogs undergoing cardiopulmonary bypass. Dogs treated with increasing doses of Oxygent survived cardiopulmonary bypass progressively better than did control animals.

Oxygent may also be beneficial as an adjunct to resuscitation. In a porcine model of near fatal haemorrhage, Oxygent treatment in addition to standard resuscitation decreased mortality from 43 to 13% [16]. Infusion of oxygenated Oxygent into the aortic arch also improved outcome in another resuscitation model [17].

Mixed venous oxygen partial pressure was higher in Oxygent-treated animals after ANH to a haemoglobin of 7 g/dl than in control animals [14,18], and measures of left ventricular systolic and

diastolic contractile function were found to be improved after Oxygent administration at a haemoglobin level of 3 g/dl [19]. This might be explained by an augmented oxygen delivery through very narrow capillaries where Oxygent particles may penetrate better than the relatively large red blood cells, and thereby improve local tissue oxygenation more than red blood cells [1]. These studies thus indicate that Oxygent indeed transports and unloads oxygen into the areas where it is needed most.

Clinical efficacy of second-generation perfluorocarbon emulsions

Oxygent has also been used in humans [6,20,21,22]. ANH to a haemoglobin concentration of approximately 9 g/dl was performed [20]. During surgery Oxygent (0.9 g/kg) was administered when a blood transfusion was deemed necessary by the anaesthesiologist, which occurred at a haemoglobin concentration of approximately 8 g/dl. Mixed venous oxygen tension and mixed venous oxygen saturation both increased significantly after Oxygent administration, and cardiac output was stable. Although only relatively little oxygen was transported by perflubron emulsion (approximately 1%), 5% of the metabolized oxygen was transported by Oxygent, again indicating that Oxygent-transported oxygen is preferentially metabolized [11,20].

Recently, the results of two large prospective randomized multicenter studies on the use of Oxygent in orthopaedic and genitourinary surgery were presented [21,22]. In the orthopaedic study [21], 147 patients undergoing hip replacement and spine surgery were haemodiluted preoperatively to a hemoglobin level of 9 g/dl. After the patients had reached a predefined transfusion trigger, they were randomized into four groups: standard of care (retransfusion of 450 ml autologous blood at an unchanged fractional inspired oxygen of 0.4); Oxygent (0.9 or 1.8 g/kg) with colloid to a total 450 ml with ventilation with an fractional inspired oxygen of 1.0; and infusion of 450 ml colloid with ventilation with an fractional inspired oxygen of 1.0. Oxygent (1.8 g/kg) was most successful in reversing transfusion triggers in 97% of patients, as compared with 60% in the control group. The duration of transfusion trigger reversal in the Oxygent 1.8 g/kg group was significantly longer (80 min) than in the control and colloid groups (55 and 30 min, respectively). In the study that including 109 patients undergoing genitourinary surgery [22], similar results were achieved. Thus, physiologic transfusion triggers may be treated at least as successfully with Oxygent as with autologous blood of colloids. This illustrates the remarkable potency of Oxygent to deliver readily available oxygen to those areas in the body in which the extra oxygen is needed most.

Future uses of perfluorocarbon emulsions

Optimal use of perfluorocarbon emulsions in the future may consist of a combination of ANH preoperatively with application of an artificial oxygen carrier such as a perfluorocarbon emulsion during the operation, a procedure termed 'augmented ANH^{SM}' [Roth DJ, Keipert PE, Faithfull NS, Zuck TF, Riess JG: Facilitated oxygen delivery in conjunction with hemodilution. US Patent #5,451,205 (issued September 19, 1995) and European Patent #EP 0627 913 B1 (issued April 4, 1998)] (Fig. 2). Augmented ANH is a concept in which patients undergo ANH to relatively low haemoglobin levels preoperatively. During the operation, when the haemoglobin concentration decreases further due to surgical blood loss and concomitant colloid or crystalloid replacement, perfluorocarbon emulsions in conjunction with 100% oxygen ventilation is administered to enhance oxygen delivery and improve tissue oxygenation. As a consequence, lower levels of haemoglobin concentration can be safely tolerated. Towards the end of the operation, the autologous blood harvested during ANH is retransfused. This will result in a relatively high haemoglobin concentration in the postoperative period and oxygen delivery will again be provided by endogenous haemoglobin. Therefore, greatly elevated arterial partial oxygen tension values are not necessary in the postoperative period and the relatively short half-life of perfluorocarbon emulsions (< 24 h) will not compromise their success in reducing allogeneic blood transfusion requirement (Fig. 2).

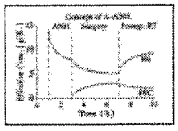

Figure 2

The concept of augmented acute normovolemic haemodilution (A-ANH) is divided into three periods (a-c). (a) Preoperative ANH with conventional volume replacement without the use of an artificial oxygen carrier such as a perfluorocarbon emulsion. (more ...)

For the concept of augmented ANH, it is important not to compromise blood coagulation during the surgical procedure [1,6], otherwise blood loss might be enhanced and thus allogeneic blood savings limited. Therefore, a careful selection of colloids is necessary to avoid blood coagulation becoming compromised [23].

Apart from the use of perfluorocarbon emulsions to reduce allogeneic blood transfusions in surgery, there are numerous other potential future indications based on their potential to augment tissue oxygenation. Such future indications will probably include treatment and prevention of cerebral ischaemia, stroke, cardiopulmonary bypass-related cerebral adverse events, spinal cord ischaemia, myocardial ischaemia due to acute infarction, percutaneous coronary angioplasty, acute limb ischaemia, emergency surgery and trauma as long as no allogeneic blood is available [21], and decompression sickness. Other applications include the use of perfluorocarbon emulsions to augment tumour oxygenation to render them more sensitive to radiation and chemotherapy, to prevent or treat sequelae of air embolism, and finally to improve organ preservation for subsequent organ transplantation (referenced in [1]).

References

1. Keipert PE. Perfluorochemical emulsions: future alternatives to transfusion. *Blood Subst Princ Meth Prod Clin Trials.* 1998;**2**:127–156.
2. Spahn DR, Leone BJ, Reves JG, Pasch T. Cardiovascular and coronary physiology of acute isovolemic hemodilution: a review of nonoxygen-carrying and oxygen-carrying solutions. *Anesth Analg.* 1994;**78**:1000–1021. [PubMed]
3. Riess JG. Overview of progress in the fluorocarbon approach to in vivo oxygen delivery. *Biomater Artif Cells Immobil Biotechnol* . 1992;**20**:183–202.
4. Riess, JG.; Keipert, PE. Update on perfluorocarbon-based oxygen delivery systems. *Blood Substitutes: Present and Future Perspectives. Edited by Tsuchida E. Lausanne: Elsevier Science SA,* 1998. pp. 91–102.
5. Keipert PE, Faithfull NS, Roth DJ, et al. Supporting tissue oxygenation during acute surgical bleeding using a perfluorochemical-based oxygen carrier. *Adv Exp Med Biol.* 1996;**388**:603–609. [PubMed]
6. Monk TG, Winston RS, Wahr JA, et al, Perflubron Emulsion Study Group. Safety and coagulation profile following administration of perflubron emulsion in patients undergoing moderate blood loss surgery [Abstract]. *Anesthesiology.* 1998;**89 (suppl)**:A397.
7. Rosoff JD, Soltow LO, Vocelka CR, et al. A second-generation blood substitute (perfluorodichlorooctane emulsion) does not activate complement during an *ex vivo* circulation model of bypass. *J Cardiothorac Vasc Anesth.* 1998;**12**:397–401. [PubMed]
8. Van De Water JM, Kagey KS, Miller IT, et al. Response of the lung to six to 12 hours of 100 per cent oxygen inhalation in normal man. *N Engl J Med.* 1970;**283**:621–626. [PubMed]
9. Tinits P. Oxygen therapy and oxygen toxicity. *Ann Emerg Med.* 1983;**12**:321–328. [PubMed]
10. Eckstein EC, Koleski JF, Waters CM. Concentration profiles of 1 and 2.5 microns beads during blood flow. Hematocrit effects. *ASAIO Trans.* 1989;**35**:188–190. [PubMed]
11. Keipert PE, Faithfull NS, Bradley JD, et al. Oxygen delivery augmentation by low-dose perfluorochemical emulsion during profound normovolemic hemodilution. *Adv Exp Med Biol.* 1994;**345**:197–204. [PubMed]

12. Hogan MC, Willford DC, Keipert PE, Faithfull NS, Wagner PD. Increased plasma O_2 solubility improves O_2 uptake of *in situ* dog muscle working maximally. *J Appl Physiol.* 1992;**73**:2470–2475. [PubMed]

13. Mosca RS, Rohs TJ, Waterford RR, et al. Perfluorocarbon supplementation and postischemic cardiac function. *Surgery.* 1996;**120**:197–204. [PubMed]

14. Habler OP, Kleen MS, Hutter JW, et al. Hemodilution and intravenous perflubron emulsion as an alternative to blood transfusion: effects on tissue oxygenation during profound hemodilution in anesthetized dogs. *Transfusion.* 1998;**38**:145–155. [PubMed]

15. Holman WL, Spruell RD, Ferguson ER, et al. Tissue oxygenation with graded dissolved oxygen delivery during cardiopulmonary bypass. *J Thorac Cardiovasc Surg.* 1995;**110**:774–785. [PubMed]

16. Stern SA, Dronen SC, McGoron AJ, et al. Effect of supplemental perfluorocarbon administration on hypotensive resuscitation of severe uncontrolled hemorrhage. *Am J Emerg Med.* 1995;**13**:269–275. [PubMed]

17. Manning JE, Batson DN, Payne FB, et al. Selective aortic arch perfusion during cardiac arrest: enhanced resuscitation using oxygenated perflubron emulsion, with and without aortic arch epinephrine. . *Ann Emerg Med.* 1997;**29**:580–587. [PubMed]

18. Habler OP, Kleen MS, Hutter JW, et al. Effects of hyperoxic ventilation on hemodilution-induced changes in anesthetized dogs. *Transfusion.* 1998;**38**:135–144. [PubMed]

19. Habler OP, Kleen MS, Hutter JW, et al. Iv perflubron emulsion versus autologous transfusion in severe normovolemic anemia: Effects on left ventricular perfusion and function. *Res Exp Med.* 1998;**197**:301–318.

20. Wahr JA, Trouwborst A, Spence RK, et al. A pilot study of the effects of a perflubron emulsion, AF on mixed venous oxygen tension in anesthetized surgical patients. *Anesth Analg.* 1996;**82**:103–107. [PubMed]

21. Spahn, DR.;van Bremt, R.;Theilmeier, G., et al. Perflubron Emulsion Study Group: Perflubron emulsion delays blood transfusion in orthopedic surgery. *Anesthesiology.* 1999.

22. Monk TG, Winston RS, Wahr JA, et al. Perflubron emulsion is more effective than blood for transfusion trigger reversal [Abstract]. *Anesth Analg.* 1998;**86 (suppl)**:S142.

23. Egli GA, Zollinger A, Seifert B, et al. Effect of progressive haemodilution with hydroxyethyl starch, gelatin and albumin on blood coagulation. An *in vitro* thrombelastography study. *Br J Anaesth.* 1997;**78**:684–689. [PubMed]

24. Looker D, Abbott-Brown D, Cozart P, et al. A human recombinant haemoglobin designed for use as a blood substitute. . *Nature.* 1992;**356**:258–260. [PubMed]